ABSTRACT

Proteolytic peptide mass mapping as measured by mass spectrometry provides an important method for the identification of proteins, which are usually identified by matching the measured and calculated m/z values of the proteolytic peptides. A unique identification is, however, heavily dependent upon the mass accuracy and sequence coverage of the fragment ions generated by peptide ionization. The present invention describes a method for increasing the specificity, accuracy and efficiency of the assignments of particular proteolytic peptides and consequent protein identification, by the incorporation of selected amino acid residue(s) enriched with stable isotope(s) into the protein sequence without the need for ultrahigh instrumental accuracy. Selected amino acid(s) are labeled with $^{13}C/^{15}N/^{2}H$ and incorporated into proteins in a sequence-specific manner during cell culturing. Each of these labeled amino acids carries a defined mass change encoded in its monoisotopic distribution pattern. Through their characteristic patterns, the peptides with mass tag(s) can then be readily distinguished from other peptides in mass spectra. The present method of identifying unique proteins can also be extended to protein complexes and will significantly increase data search specificity, efficiency and accuracy for protein identifications.